Patterns of Sex Change of the Protandric Patellacean Limpet *Lottia gigantea* (Mollusca: Gastropoda)

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Abstract. Experiments were done to test the effects of density and age on the probability of sex change in the protandrous limpet *Lottia gigantea* (Sowerby, 1834). Because of tag loss and mortality, final numbers of individuals in each experiment were low. However, some trends are present in these data that are distinctive. Young, territorially subordinate limpets, transplanted to large, isolated, empty territories, had a low proportion of sex changers during the first year of the experiment (4/22), but a significantly higher proportion during the second year (9/11). Limpets maintained at higher densities had a low proportion of sex changers during both the first (1/7) and second (1/5, 1/7, 1/12) years after transplantation, regardless of age. These data suggest that either low density promotes sex change with a one year lag period, or that high density inhibits sex change that would otherwise occur when the limpets are 2 to 3 years old. The presence of an inherent probability of sex change cannot be ruled out.

INTRODUCTION

SEX-CHANGING MARINE invertebrates have received considerable attention over the last few years (see review by POLICANSKY, 1982). Most of the documented cases involve protandry (male → female) and several scenarios and models have been proposed to explain its selective advantage or adaptive significance (GHISELIN, 1974; WARNER, 1975; CHARNOV & BULL, 1977).

Protandry has often been investigated in the Mollusca, and occurs in a diverse group of taxa within the phylum (POLICANSKY, 1982). Taxa with copulatory structures are usually the subjects of these investigations because changes in sex are readily detected by examining external structures (COE, 1944). However, the development of a technique allowing direct sampling of the gonadal contents of suspected protandric species that lack external characters (*e.g.*, patellacean limpets) (WRIGHT & LINDBERG, 1979) enabled us to monitor directly protandry in the limpet *Lottia gigantea* (Sowerby, 1834) (WRIGHT & LINDBERG, 1982).

In this paper we report results of initial experiments to determine the ecological and interactive factors influencing sex change in *L. gigantea*. Although our results are not entirely conclusive, we present them because these data suggest a complicated pattern of sex change involving ecological factors, lag periods, and possibly genetic probabilities of sex change within the population. It is our intention to bring these data to the attention of other workers so that possible antagonistic or synergistic effects of these phenomena are not confused or overlooked in other studies.

MATERIALS AND METHODS

Lottia gigantea is a large, territorial, intertidal limpet, sometimes reaching lengths over 100 mm (STIMSON, 1970). Larger individuals occupy either isolated territories (usually surrounded by sessile invertebrates such as barnacles and mussels) (Figure 1) or contiguous territories (where they occur at such high density that territorial boundaries are impossible to discern) (Figure 2). Younger, smaller
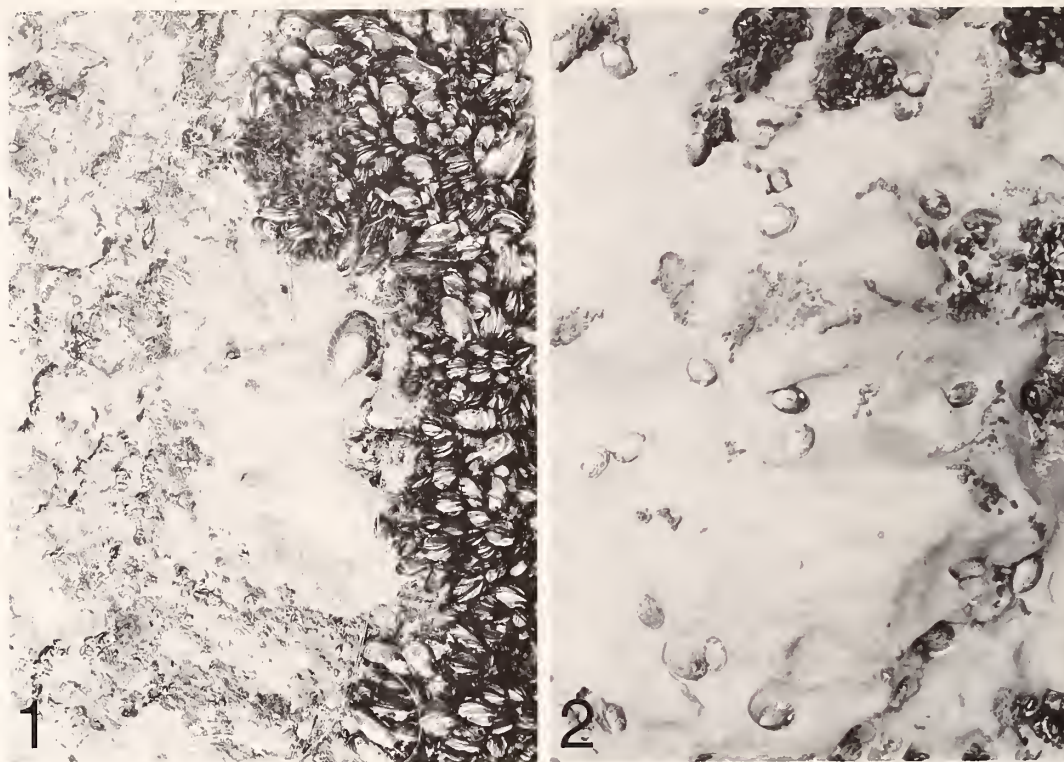
Explanation of Figures 1 and 2

Figure 1. Low density experimental treatment on San Nicolas Island, California.
Figure 2. High density experimental treatment on San Nicolas Island, California.

individuals typically graze on the territories of larger in-dividuals and respond to contact with the resident limpet by escaping to the perimeter of the territory before the resident can respond aggressively (WRIGHT, 1982). Most limpets can be categorized behaviorally as either intruders or residents; intruders are always evasive while residents are usually aggressors (WRIGHT, 1982).

The growth of *Lottia gigantea* is relatively indetermi-nate; although each habitat imposes a maximum size above which no limpet can grow, that size ranges from 40 mm to above 70 mm depending on characteristics of the habitat (*i.e.,* density of other grazing herbivores, intertidal height, wave exposure, substratum, *etc.*). In addition, individual growth varies inversely with size relative to the local max-imum (Wright, unpublished data). Thus, a 40-mm limpet in a local population where the maximum size is near 40 mm is growing slowly, if at all, and generally will be older than a 40-mm limpet in a population where the maximum size is nearer to 70 mm. We quickly realized that older, slower growing limpets could be recognized by their heavily eroded shells (Figure 4) while younger, fast growing lim-pets could be recognized by the checkerboard pattern of

their shells (Figure 3). Using these criteria, we could roughly judge the relative age of *L. gigantea* by external appearance within a habitat.

Experimental manipulations of *Lottia gigantea* were done on San Nicolas Island, Ventura County, California (33°16′N, 119°30′W) between December 1980 and De-cember 1982. In all treatments, the experimental limpets were removed from the substratum while moving (to avoid trauma) and sexed by sampling the gonad through the rear body wall with a hypodermic syringe (WRIGHT & LINDBERG, 1979). The dorsal surface of the shell was marked with a plastic number embedded in waterproof epoxy before the limpet was placed in either a low (Figure 1) or high density (Figure 2) setting.

The first experiment, begun in December 1980, tested the hypothesis that high density lowers the probability of sex change. The experiment was performed with 35 males, all of which were observed to exhibit evasive behavior. Of these 35, 26 were placed on isolated empty territories (low density) where food supply was abundant and detrimental interactions with other *Lottia gigantea* nil. Seven males were placed in a high density setting of limpets with in-
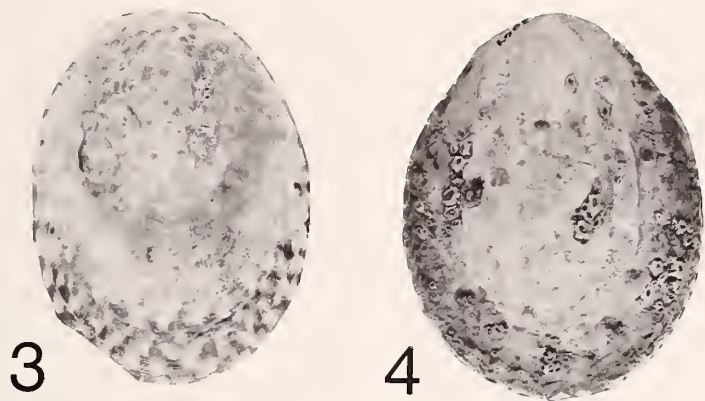
Explanation of Figures 3 and 4

Figure 3. *Lottia gigantea.* Young, checkerboard specimen; length = 55 mm.

Figure 4. *Lottia gigantea.* Old, eroded specimen; length = 60 mm.

discernable boundaries. In such settings, food would be sparse and interactions frequent and potentially detrimental.

The second experiment, begun in December 1981, was designed to test the effect of age on the probability of sex change. We selected 35-mm to 45-mm 'old' and 'young' males by using the shell erosion criterion discussed above. We placed all of these in a high density treatment identical to the high density treatment in the first experiment.

At the end of each year (December) the gonadal contents of the limpets were sampled. Limpets that had become female were removed from the experimental settings and dissected to verify sex change and to check for possible

simultaneous hermaphroditism. Males were replaced in their experimental positions.

RESULTS

The results of the high and low density experiments are shown in Figure 5 and Table 1. Also included in the figure are data for *Lottia gigantea* sex change from WRIGHT & LINDBERG (1982).

The proportion of males changing sex during the first year was low in all treatments (0.08 to 0.15) and did not appear to be affected by density or age (χ^2, $P > 0.05$). During the second year, 9 out of 11 in the low density treatment changed sex versus only 1 out of 5 in the high density treatment. These were not significantly different proportions (Fisher's exact text, $P = 0.071$) perhaps due to the low numbers in the high density treatment. In fact, the only significant difference throughout all treatments was between the limpets in the low density treatment at
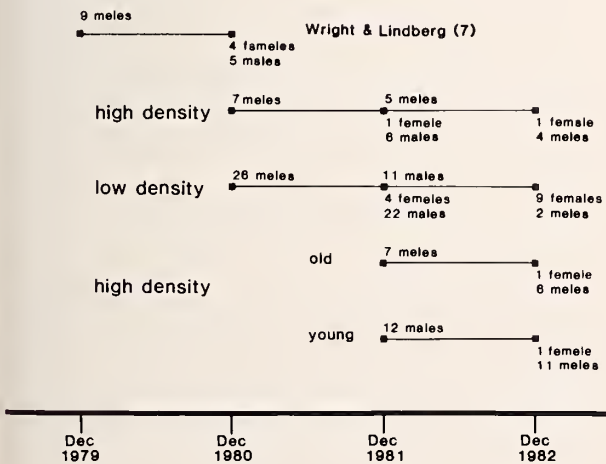

Figure 5

Patterns of sex change in *Lottia gigantea.* Number of males monitored during the experiment appears above the line, results below the line. ■ = monitoring times, (7) = (1982).

Table 1

Percent and number of male *Lottia gigantea* changing sex in various treatments. Lines connecting treatment percentages indicate significant differences at the 0.05 level or greater (Fisher's exact test). All other proportions not significantly different ($P \geq 0.05$).

Treatment	Percent changing sex and (n's)	
	First year	Second year
High density (HD)	14% (1/7)	20% (1/5)
HD Old	14% (1/7)	—
HD Young	8% (1/12)	—
Low density	15% (4/26)	82% (9/11)

the end of two years, and any of treatments at the end of one year.

No simultaneous hermaphrodites were found.

DISCUSSION

Formally, our data support nothing more than the statement that "limpets in the second year of a sex change experiment change sex more than during the first year." There are two interpretations of this conclusion. (1) The limpets that were used in the density experiment (both treatments) were all of similar age and sex change is age-specific. (2) Limpets respond to changes in environmental conditions (*i.e.,* abundant food, no agonistic losses) by changing sex, but there is a lag period greater than 12 months but less than 24 months between environmental change and sex change. We favor the second interpretation for several reasons. First, based on a preliminary aging technique, there does not seem to be any single age for sex change (Wright, personal observation). Males can be found that are probably 5 years or older, and females can be found that are probably no more than 1½ years old. Secondly, the results of the high density, young versus old experiment (Figure 5, Table 1) argue against the presence of an age-specific sex change phenomenon. Finally, the proportion of sex changers during the second year in low (9/11) versus high (1/5) density treatments, although not statistically significant, is highly disproportional—a fact that cannot stand alone given statistical convention, but that can tip the scales of a close argument. Thus, we favor the second hypothesis and the presence of environmental sex determination in *Lottia gigantea.*

In addition to the evidence supporting environmental sex determination in *Lottia gigantea,* there is a second intriguing pattern present in the data. This is the proclivity of about 15% of the population to change sex annually regardless of environmental setting or age (Table 1). This pattern may represent a genetically programmed sex change component that is virtually immune to environmental factors. Thus, sex change in *L. gigantea* may be regulated by two mechanisms: (1) environmental sex determination and (2) genetically programmed sex change. Another explanation for the persistent low proportion of sex changers in our treatments is that the limpets that changed had experienced an environmental release the year before they were used in the experiment. A final possibility is that the sex changers were simply responding to variations within the two treatments. Although we cannot rule out any of these possibilities, it is unlikely that all of the treatments we set up in December of 1980 and again a year later in December of 1981 all received similar proportions of these predisposed changers and/or had similar environmental variabilities within the study sites.

Neither environmental nor genetically programmed sex determination was suggested by the results of our first experiment (WRIGHT & LINDBERG, 1982; Figure 5 herein). We believe that the high proportion of sex chang-ers in our first experiment (4/9) resulted because we confounded limpets with different histories. Although we carefully chose limpets that were about the same size, we did not know the territorial status of the limpets, and therefore, our experiment undoubtedly contained both intruders and residents. Thus, some of the males that changed sex may have been in low density situations for as much as a year prior to our using them in the experiment, and our results therefore include both environmentally determined sex changers that were programmed to change sex the year before, as well as limpets that were genetically programmed to change sex that year. Because of this complication, we do not feel that the earlier data set can be combined or compared with the data set presented herein.

In most previously studied protandrous marine invertrates (see review by HOAGLAND [1978]) sex change is predominantly genetically regulated or environmentally determined. In the echiuroid genus *Bonellia* both environmental sex determination and genetic sex determination appear to be important (GOULD-SOMERO, 1975; LEUTERT, 1975). In *Bonellia* the two different mechanisms of sex change are thought to represent two different genotypes in the population. The first contains "true" males and females that are genetically determined and do not change sex; the second contains a genotype in which sex is environmentally determined.

Scenarios to explain protandry in patellacean limpets have relied exclusively on genetic interpretations, including rather elaborate hypotheses with two or more genotypes in the population, representing true males and females and individuals changing sex at different ages (MONTALENTI & BACCI, 1951). Recent analysis of static sample data for *Patella vulgata* in England has identified an age-specific property associated with sex change (BALLANTINE, 1961; CHARNOV, 1982) and BRANCH (1974a) has presented data for *Patella oculus* (Born, 1778) from South Africa that strongly suggest an age-specific genetic mechanism. However, for *Lottia gigantea* and many other species of patellacean limpets age- and/or size-specific sex change and its resulting sex ratios are less apparent in static samples. Typically there are both small females and large males present in these samples. Moreover, the switch in the sex ratio from predominantly males to predominately females is not sharp, but spread over several age or size classes (BRANCH, 1974b; WRIGHT & LINDBERG, 1982).

The tendency for workers to propose multiple genotypes for some protandrous species may result from an observation bias. In studies of species in which sex change can be externally observed (*e.g.,* in the Calyptraeidae) sex change is often found to be a response to environmental change. However, there has been no such conclusion for molluscan species in which gender can be determined only once. Instead, genetic control alone is postulated to be responsible for sex change. Workers on this latter group of species have had to complicate their genetic hypotheses in order to explain the apparently large variation in the

timing of sex change; such variation is inferred from the wide distribution of sexes as a function of size. Thus, they must further hypothesize the existence of one or more additional genotypes that change sex at different ages and/or a genotype that does not change sex at all. Missing from these discussions is the possibility of environmental sex determination, in spite of its utility in explaining highly variable size of sex change (*i.e.,* a highly variable or complex environment). Based on the results presented here, we can rule out to some extent the importance of the different-genotype hypothesis because under no conditions would it predict that such a high proportion of limpets as 9 out of 11 would change sex.

We have presented data suggesting that environment (including the social interactions among conspecifics) can control to some degree when sex change occurs in *Lottia gigantea*. We believe that our experiments suggest environmental control as an alternative hypothesis to multiple genotypes in explaining the causes of sex change in other patellacean limpets, especially when the limpet shows territoriality and/or other indications of profound environmental and/or social changes during its ontogeny (BRANCH, 1974b, 1975).

The ultimate cause of sex change in patellacean limpets is hormonal (CHOQUET, 1969). We have attempted to identify and elucidate proximate causes that can result in the patterns of sex change seen in our experimental manipulations and in static population samples. Although preliminary, we present them because they indicate that mechanisms could be overlooked if experimental manipulations are not followed for at least two years or if the past histories of the manipulated animals are not known or at least limited (*i.e.,* presence of evasive behavior in *Lottia gigantea*). Moreover, our data suggest that two mechanisms (environmental sex determination and underlying genetic determination) may be present. Such multiple effects can produce results that are difficult to interpret in static samples, short term experiments, or in traditional paradigms, and may ultimately lead to an incorrect interpretation of the mechanisms or an underestimation of the processes controlling sex change.

ACKNOWLEDGMENTS

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